

In the Claims

1. (Currently amended) A DNA construct comprising:

- a) at least one a first nucleic acid sequence containing the nucleotide sequence coding for at least one respective a product of interest;
- b) a second nucleic acid sequence containing the nucleotide sequence coding for a dimerization domain; and
- c) a third nucleic acid sequence containing the nucleotide sequence coding for *E. coli* α -hemolysin (HlyA) or for a fragment of said protein comprising the recognition signal of the *E. coli* Hly transport system secretion mechanism, or a nucleotide sequence coding for a homologous gene, or a nucleotide sequence coding for a natural or artificial variant of HlyA or of a fragment thereof comprising the recognition signal of the *E. coli* Hly transport system secretion mechanism; wherein the 3' end of said first nucleic acid sequence is bound to the 5' end of said second nucleic acid sequence, and the 3' end of said second nucleic acid sequence is bound to the 5' end of said third nucleic acid sequence.

2. (Cancelled)

3. (Currently amended) The DNA construct according to claim 1, wherein said product of interest is chosen from enzymes, enzymatic inhibitors, hormones, molecules involved in cell adhesion, molecules involved in ~~and/or~~ signaling, molecules involved in detection, molecules involved in ~~or~~ labeling, molecules made up of domains, immunogenic antigens, therapeutic agents, ~~and~~ or immunoregulating molecules.

4. (Currently amended) The DNA construct according to claim 1 ~~3~~, wherein said product of interest is chosen from tumor-specific antigens, auto-immune disease antigens, growth factors, cytokines,

interleukins, interferons, ~~and~~ or miniantibodies.

5.-7. (Cancelled)

8. (Currently amended) The DNA construct according to claim 1, wherein said third nucleic acid sequence is chosen from:

- a) ~~the~~ a nucleotide sequence coding for HlyA of *E. coli*;
- b) a nucleic acid sequence comprising the nucleotide sequence coding for the last 60 amino acids of the C-terminal end of HlyA of *E. coli*;
- c) a nucleic acid sequence made up of a nucleotide sequence coding for the last 60 amino acids of the C-terminal end of HlyA of *E. coli*;
- d) ~~the~~ a nucleotide sequence identified as SEQ ID NO: 1; ~~and~~ or
- e) a nucleotide sequence coding for the amino acid sequence identified as SEQ ID NO: 2.

9.-11. (Cancelled)

12. (Currently amended) The DNA construct according to claim 1, further comprising a ~~fifth~~ nucleic acid sequence coding for a polypeptide susceptible of being used for isolation or purification purposes.

13-14. (Cancelled)

15. (Currently amended) The DNA construct according to claim 1, further comprising a ~~sixth~~ nucleic acid sequence coding for a peptide susceptible of being used for recognition purposes.

16-21. (Cancelled)

22. (Currently amended) The DNA construct according to claim 1, further comprising a ~~seventh~~ nucleic acid sequence comprising a

nucleic acid sequence coding for an amino acid sequence susceptible of being cleaved specifically by enzymatic or chemical means.

23.-27. (Cancelled)

28. (Currently amended) An expression cassette comprising a DNA construct according to claim 1 ~~any of the previously claims~~
~~operatively bound to an expression control sequence.~~

29-30. (Cancelled)

31. (Currently amended) A vector comprising at least one DNA construct according to claim 1 ~~any of claims 1 to 27 or at least one~~
~~the expression cassette according to any of claims 28-30.~~

32.-34 (Cancelled)

35. (Currently amended) A gram-negative bacteria comprising at least one DNA construct according to claim 1, ~~any of claims 1 to 27,~~
~~or at least one expression cassette according to any of claims 28-30,~~
~~or at least one vector according to any of claims 31 to 33, wherein~~
the at least one DNA construct is included in a vector or expression
cassette.

36.-37. (Cancelled)

38. (Currently amended) A dimeric fusion protein obtainable by expression of the nucleic acid sequences contained in a DNA construct according to claim 1 ~~any of claims 1 to 27, or at least one expression~~
~~cassette according to any claims 28-30, or at least one vector~~
~~according to any claims 31 to 33.~~

39. (Currently amended) The Fusion protein according to ~~claims~~
~~37-38~~ claim 38, wherein each monomer comprises:

- (i) ~~the~~ a amino acid sequence of a product of interest;
- (ii) an amino acid sequence corresponding to a dimerization domain; and
- (iii) ~~the~~ a amino acid sequence of α -hemolysin (HlyA) of *Escherichia coli* or of a fragment of said protein comprising the recognition signal of the *E. coli* hemolysin (Hly) transport system secretion mechanism.

40. (Currently amended) The Fusion protein according to claim ~~40~~ 39, wherein each monomer comprises:

- (i) a product of interest chosen from an enzyme, an enzymatic inhibitor, a hormone, a molecule involved in cell adhesion and/or signaling, molecules involved in detection or labeling, molecules made up of domains, an immunogenic antigen, a therapeutic agent, or an immunoregulating molecule;
- (ii) a dimerization domain chosen from a peptide helix ~~and~~ or a coiled coil structure; and
- (iii) ~~the~~ an entire ~~whole~~ *E. coli* HlyA amino acid sequence, or an *E. coli* HlyA fragment comprising the recognition signal of the *E. coli* Hly transport system secretion mechanism.

41.- (Currently amended) The Fusion protein according to claim 39 ~~41~~, wherein each monomer comprises:

- (i) a product of interest chosen from a tumor-specific antigen, an auto-immune disease antigen, a growth factor, a cytokine, an interleukin, an interferon ~~and~~ or a miniantibody;
- (ii) a dimerization domain chosen from a peptide helix ~~and~~ or a coiled coil structure; and
- (iii) an entire ~~the whole~~ *E. coli* HlyA amino acid sequence, or an *E. coli* HlyA fragment comprising the recognition signal of the *E. coli* Hly transport system secretion mechanism.

42.- (Currently amended) The Fusion protein according to ~~claims~~ 37-41 claim 39, wherein each monomer further comprises at least one member selected from the group consisting of (a) ~~a spacer peptide between the product of interest and the dimerization domain; and/or~~ (b) ~~a peptide to facilitate the isolation and purification of the peptide or fusion protein; and/or~~ (c) (b) a peptide which allows recognition of the peptide or fusion protein; ~~and/or~~ (d) and (c) an amino acid sequence susceptible of being cleaved specifically by enzymatic or chemical means.

43. (Cancelled)

44. (Currently amended) A method for producing a product of interest in the form of a dimeric fusion protein according to claim 38, wherein the method comprises: ~~any of claims 37 to 43, comprising growing a bacteria according to claims 34-36~~ under conditions allowing the production and excretion of said product of interest to the culture medium in the form of a dimeric fusion protein.

45. (Currently amended) The method ~~Method~~ according to claim 44 for producing a dimeric fusion protein, comprising two products of interest.

46. (Cancelled)

47.- (Currently amended) The DNA construct according to claim 1, wherein the DNA construct is used in the ~~Use of a DNA construct according to claims 1 to 27 for the creation of a dimeric protein library, wherein the protein library may be used choosing molecules with the capacity to bind to a given antigen.~~

48. (Cancelled)

49. (Currently amended) The Fusion protein according to claim 39,

~~claims 37 to 43, wherein the fusion protein is used for the use thereof in therapy of an ailment responsive to the product of interest, or for use in in vitro or in vivo diagnosis of such ailment.~~

50.- (Cancelled)

51. (New) A DNA construct comprising:

- a) a first nucleic acid sequence containing the nucleotide sequence coding for a product of interest;
- b) a second nucleic acid sequence containing the nucleotide sequence coding for a dimerization domain;
- c) a third nucleic acid sequence containing the nucleotide sequence coding for *E. coli* α -hemolysin (HlyA) or for a fragment of said protein comprising the recognition signal of the *E. coli* Hly transport system secretion mechanism, or a nucleotide sequence coding for a homologous gene, or a nucleotide sequence coding for a natural or artificial variant of HlyA or of a fragment thereof comprising the recognition signal of the *E. coli* Hly transport system secretion mechanism; and
- d) a fourth nucleic acid sequence coding for a spacer peptide located between said first and second nucleic acid sequences, wherein the 5' end of said fourth nucleic acid sequence is bound to the 3' end of said first nucleic acid sequence, and the 3' end of said fourth nucleic acid sequence is bound to the 5' end of said second nucleic acid sequence and wherein the 3' end of said second nucleic acid sequence is bound to the 5' end of said third nucleic acid sequence.

52. (New) An expression cassette according to claim 28, further comprising an additional nucleic acid sequence selected from a group consisting of:

- a) a nucleic acid sequence coding for a polypeptide susceptible

of being used for isolation or purification purposes;

b) a nucleic acid sequence coding for a peptide susceptible of being used for recognition purposes; and

c) a nucleic acid sequence comprising a nucleic acid sequence coding for an amino acid sequence susceptible of being cleaved specifically by enzymatic or chemical means.

53. (New) The vector according to claim 31, wherein the product of interest is selected from the group consisting of enzymes, enzymatic inhibitors, hormones, molecules involved in cell adhesion and/or signaling, molecules involved in detection or labeling, molecules made up of domains, immunogenic antigens, therapeutic agents, immunoregulating molecules, tumor-specific antigens, auto-immune disease antigens, growth factors, cytokines, interleukins, interferons, and miniantibodies; and wherein the third nucleic acid sequence is a member selected from the group consisting of:

a) a nucleotide sequence coding for HlyA of *E. coli*;

b) a nucleic acid sequence comprising the nucleotide sequence coding for the last 60 amino acids of the C-terminal end of HlyA of *E. coli*;

c) a nucleic acid sequence made up of a nucleotide sequence coding for the last 60 amino acids of the C-terminal end of HlyA of *E. coli*;

d) a nucleotide sequence identified as SEQ ID NO: 1; and

e) a nucleotide sequence coding for the amino acid sequence identified as SEQ ID NO: 2.

54. (New) A vector comprising at least one DNA construct according to claim 53.

55. (New) A gram-negative bacteria comprising at least one DNA construct according to claim 53.

56. (New) A dimeric fusion protein obtainable by expression of

the nucleic acid sequences contained in a DNA construct according to claim 53.

57. (New) The DNA construct according to claim 51, further comprising;

a) a fifth nucleic acid sequence coding for a polypeptide susceptible of being used for isolation or purification purposes;

b) a sixth nucleic acid sequence coding for a peptide susceptible of being used for recognition purposes; and

c) a seventh nucleic acid sequence comprising a nucleic acid sequence coding for an amino acid sequence susceptible of being cleaved specifically by enzymatic or chemical means.

58. (New) A dimeric fusion protein obtainable by expression of the nucleic acid sequences contained in a DNA construct according to claim 51.

59. (New) The fusion protein according to claim 58, wherein each monomer comprises:

- (iv) a product of interest chosen from an enzyme, an enzymatic inhibitor, a hormone, a molecule involved in cell adhesion and/or signaling, molecules involved in detection or labeling, molecules made up of domains, an immunogenic antigen, a therapeutic agent, or an immunoregulating molecule;
- (v) a dimerization domain chosen from a peptide helix or a coiled coil structure; and
- (vi) an entire *E. coli* HlyA amino acid sequence, or an *E. coli* HlyA fragment comprising the recognition signal of the *E. coli* Hly transport system secretion mechanism.